# Dapsone Suppresses Integrin-Mediated Neutrophil Adherence Function

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The anti-inflammatory influence of dapsone may involve suppression of neutrophil chemotaxis to selected attractants, but other actions of the drug are likely also involved. We have discovered that dapsone may suppress migration of neutrophils to extravascular sites through inhibition of adherence functions required for neutrophil recruitment. Neutrophil adherence mediated by integrins (CD11/CD18 or Mac-1 family receptors) was measured in vitro in terms of binding of stimulated cells to albumin-coated wells of microtiter plates, using phorbol myristate acetate (PMA) and N-formylmethionyl-leucyl-phenylalanine (FMLP) as stimuli. Adherence was assessed by staining attached cells with crystal violet dye and measuring the dye concentration at $OD_{590}$ using an automated plate reader. The role of integrins in this assay was confirmed by the ability of anti-integrin antibody to suppress stimulated neutrophil adherence. The $OD_{590}$ value for cells adhering to albumin in the absence of stimulus and dapsone averaged $0.2 \pm 0.04$ (SEM) over five experiments. In the presence of 0.1 $\mu$M PMA or $10^{-6}$ M FMLP, the $OD_{590}$ values averaged $0.88 \pm 0.1$ and $0.75 \pm 0.12$, respectively. Dapsone did not affect unstimulated neutrophil adherence but, when present with stimulus, produced a dose-related inhibitory effect on adherence. Fifty percent inhibitory doses were approximately 150 $\mu$g/ml dapsone for both stimuli. Sulfapyridine reproduced the inhibitory effect of dapsone, but two structurally related compounds, hydrochlorothiazide and furosamide, did not. The observed ability of dapsone to inhibit neutrophil chemotaxis under agarose to FMLP and interleukin-8 may also be explained by interference with integrin-mediated adherence required for motility in this assay system. To consider if dapsone might have a similar inhibitory influence on neutrophil adherence in vivo, we tested the stimulated adherence function of neutrophils isolated from three individuals on dapsone therapy for dermatitis herpetiformis. Stimulated adherence of patients' cells averaged less than 40 percent of the control value. Suppression of leukocyte integrin function may therefore also contribute to the ability of dapsone to inhibit neutrophil infiltration in neutrophilic dermatoses. *J Invest Dermatol 98:135–140, 1992*

Dapsone (4,4′-diaminodiphenyl sulfone) as an anti-inflammatory agent is a drug of choice for management of dermatitis herpetiformis (DH) and a number of other non-infectious diseases having in common the presence of neutrophils or eosinophils as the preponderant infiltrating cell [1]. The mechanism(s) involved in the anti-inflammatory influence of dapsone and other sulfones has been related to suppression of leukocyte chemotactic and cytotoxic functions.

Studies of in vitro effects of dapsone on neutrophils suggest that dapsone inhibits i) chemotaxis [2–4]; ii) activities of myeloperoxidase [2,5–7] and other lysosomal enzymes [8]; and iii) production of toxic oxygen intermediates [9,10]. Although suppression of any of these functions could blunt inflammation, only suppression of chemotaxis would reduce neutrophil infiltration into lesional skin. This effect would explain reduction of neutrophil infiltration in vivo in the presence of IgA deposition [11] and complement activation [12], but dapsone has not been found to suppress neutrophil chemotaxis in vitro to leukocyte-derived chemotactic factor [3] or the complement-derived attractant C5a [2,3,6,13]. Either these in vitro tests of neutrophil chemotaxis do not faithfully reproduce dapsone-mediated suppression of chemotaxis in vivo, or dapsone has an inhibitory influence on some other cell function critical for neutrophil recruitment, i.e., adherence.

Our studies show that dapsone reduces a neutrophil adherence function in vitro related to recruitment to extravascular tissue sites. Attachment of leukocytes to vascular endothelial cells is an essential first step in this process, providing for both targeting of cells to specific sites and the adherence required for cell motility. Two major subgroups of leukocyte adherence receptors play complementary roles in leukocyte recruitment; "selectins" provide for recognition of inflammatory sites, and "integrins" mediate adherence reac-

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Abbreviations:

- CD11/CD18: "cluster designation" notation for the alpha and beta subunits of leukocyte integrins, respectively
- DH: dermatitis herpetiformis
- FMLP: synthetic tripeptide neutrophil chemoattractant, N-formyl-methionyl-leucyl-phenylalanine
- LFA-1: leukocyte function antigen 1 is a second of three leukocyte integrins; mediates immunologic interactions
- $LTB_4$: leukotriene $B_4$, a neutrophil chemoattractant
- Mac-1 receptor: one of three leukocyte integrins; mediates binding of complement-(iC3b-) opsonized and cell-matrix interactions
- p150,95: third of three leukocyte integrins; name denotes respective sizes of the alpha and beta peptides
- PBS: phosphate-buffered saline
- PMA: phorbol myristate acetate, a neutrophil agonist

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tions essential for cell migration through the vascular wall and into the extravascular tissues (reviewed in [14,15]).

The leukocyte integrins are represented by a family of three glycoproteins known as LFA-1, Mac-1, and p150,95 [14,15]. Each member of the Mac-1 family contains a unique alpha-subunit (CD11a, CD11b, and CD11c, respectively) and a common beta-subunit (CD18) in non-covalent association. The functional importance of the leukocyte integrins is best demonstrated by the inability of patients with congenital leukocyte adhesion deficiency (LAD) to form pus in response to infection [16]. Mutations in the beta subunit results in the absence of functional integrins on neutrophils from LAD patients, rendering these cells unable to migrate in response to chemoattractants in vitro, and reach sites of infection in vivo. The ability of anti-beta subunit antibody to suppress the same functions of control neutrophils in vitro [17] and in vivo [18–20] further supports a role for these receptors in neutrophil recruitment. An inhibitory influence of dapsone on integrin-mediated neutrophil adherence could therefore effectively limit the accumulation of neutrophils at lesional sites in DH.

We will show that dapsone interferes with neutrophil chemotaxis and adherence in vitro, using integrin-dependent assays of these functions, and that dapsone therapy suppresses integrin-mediated adherence of patient neutrophils measured ex vivo. These findings lead us to propose that the anti-inflammatory property dapsone may reflect an anti-adherence property of this agent.

## MATERIALS AND METHODS

**Reagents** Dapsone, sulfapyridine, sulfanilamide, furosemide, hydrochlorothiazide, dimethyl sulfoxide (DMSO, ACS reagent grade), phorbol myristate acetate (PMA), bovine serum albumin (BSA), and N-formylmethiolyl-leucyl-phenylalanine (FMLP) were obtained from Sigma Chemical Co., St. Louis, MO. Phosphate-buffered saline (PBS) and minimal essential medium (MEM) were from Biologos, Naperville, IL. Interleukin-8 (IL-8) was from Genzyme, Boston, MA. Microtiter plates for the adherence assay were from Corning Glass Works (type 25860), Corning, NY, and tissue culture dishes for the chemotaxis assay were from Becton Dickenson Labware (Falcon 3002), Oxnard, CA. Mono-Poly Resolving Medium was from Flow Labs, McLean, VA.

**Solubilization of Dapsone** Dapsone, sulfapyridine, furosemide and hydrochlorothiazide were dissolved in DMSO and diluted with MEM to final working concentrations. The concentration of DMSO present with the highest dose of any reagent was 0.5%.

**Isolation of Neutrophils** Heparinized peripheral blood (10 U/ml) was obtained following informed consent from volunteer control and patient subjects. Neutrophils were isolated by density centrifugation. Cells from the lower band were recovered and washed once in $Ca^{++}/Mg^{++}$-free PBS. Contaminating erythrocytes were eliminated by hypotonic lysis. The neutrophils were washed once again in PBS and resuspended in MEM with $Ca^{++}/Mg^{++}$ at a concentration appropriate for the adherence or chemotaxis assay. Dapsone was included in the suspension medium for chemotaxis studies at the doses indicated. Cells were counted using an electronic cell counter.

**Measurement of Chemotaxis** Neutrophil random and chemotactic motility functions were measured using the under agarose technique previously described [21], except that dapsone was included in the agarose at the doses matching those used for cell suspension. FMLP was used as chemoattractant at a dose of $5 \times 10^{-7}$ M: IL-8 was used at $1 \times 10^{-6}$ M. The incubation time was 2 h, and motility functions were measured as the projected distance (40×) of migration from the well margin to the leading front of the migration pattern.

In brief, this assay involves the filling of 60 × 15 mm tissue culture dishes with 6 ml of fluid agarose-gelatin. After gelation, six pairs of wells, 3 mm in diameter and spaced 3 mm apart (edge to edge), are then cut in a radial pattern. Neutrophils are added to the inner series of wells and chemoattractant to the outer series. During incubation the neutrophils migrate radially into the space between the surface of the culture dish and the agarose gel. Flooding of the dish with glutaraldehyde fixes the cells to the plate surface to allow removal of the gel and staining of the migrated cells. The migrated cells form an oblong pattern due to differences between distances of migration toward and away from the well containing the attractant. Cells migrating toward the attractant well will move a greater distance outward than cells migrating from the opposite side of the inner well, due to the directional versus random qualities of cells moving in the respective directions. Migration of neutrophils in any system requires their adherence interaction with substrate; in the under-agarose system this adherence is mediated primarily by integrin receptors [17].

Neutrophil chemotaxis measured by the membrane filter method involves counting of cells that have moved across a filter separating an upper compartment containing cells in culture medium from a lower compartment containing the attractant. Migration of neutrophils in the membrane filter material is not dependent upon integrin-mediated adherence [17].

**Measurement of Adherence** Neutrophil adherence was measured by a "micro" method using wells of microtiter plates coated with albumin as substrate, FMLP or PMA to stimulate adherence, and crystal violet dye to stain adherent cells. Albumin was selected as substrate because human neutrophils bind to polystyrene beads coated with albumin through Mac-1 family receptors [17], and because others have used albumin-coated plastic to measure this neutrophil adherence function [22]. The role of Mac-1 family receptors in neutrophil adherence to albumin-coated microtiter plates was further confirmed using a murine monoclonal anti-CD18 antibody (Ab-1) obtained from Oncogene Science, Manhasset, NY. Selection of FMLP and PMA as stimuli for adherence was also based upon an earlier report [17]. Staining of adherent cells with crystal violet dye was based upon a colorimetric assay for tumor necrosis factor cytotoxicity [23].

Plates were coated with substrate by adding 100 μl of 0.5 gm% in PBS to each well, incubating for 60 min at 37°C, and washing 3× with 100 μl PBS per well. Each well then received 100 μl MEM or MEM containing drug, 100 μl of neutrophils ($2 \times 10^6$ ml in MEM), and 10 μl of PMA (2 μM in PBS) or FMLP ($2 \times 10^{-5}$ M in PBS). Following incubation for 60 min at 37°C, the wells were aspirated with a Nunc Immuno Wash 12-plate washer (Nunc, Inc., Naperville, IL), quickly and gently filled with PBS from a wash bottle, and aspirated again with the washer. Before drying, each well received 50 μl of staining solution. The plate was then incubated for 10 min at room temperature and the wells washed with tap water until no more dye appeared to come off the plate. After blotting the plate on paper towels, 100 μl of 1% sodium dodecyl sulfate was added to each well to release the dye associated with the adherent cells. The plate was rocked gently to mix the solubilized dye and read at 590 nm on a UVMax Microplate Reader (Molecular Devices, Menlo Park, CA). The staining solution contained 0.5 g % crystal violet dye, 3% (v/v) formaldehyde, and 30% (v/v) ethanol in PBS.

**Dermatitis Herpetiformis Patients** Neutrophils for adherence assays were isolated from three patients receiving 50 to 200 mg dapsone per day as treatment for DH.

**Data Summary and Statistical Analysis** All experiments were performed at least three times using cells isolated from as many different donors, and each test was replicated three times within each experiment. Summary data represent the means and standard error values derived from the mean values for each experimental series. Statistical analyses used the Dunnett t test for multiple comparisons with a control value.

## RESULTS

**Effect of Dapsone on Neutrophil Chemotaxis Under Agarose** We initiated our studies of dapsone to resolve the discrepant findings reported for dapsone as an inhibitor of chemotaxis in the agarose and membrane filter systems. Reports by Anderson et al [2]

and by Harvath et al [3] cite an inhibitory influence of dapsone on neutrophil chemotaxis to the synthetic tripeptide FMLP measured using membrane filter systems, but Millar et al [24] found no effect of dapsone on chemotaxis to FMLP assayed under agarose. We considered that these variable results might be due to differences in reagent concentration and the assay method used. Dapsone was used at concentrations of 30, 100, and 300 $\mu$g/ml in our protocol and, because diffusion may quickly reduce the concentration of drug added with the cells, dapsone was included in both the cell-suspension medium and the agarose gel. The effects of dapsone on chemotaxis to IL-8 and random motility were also evaluated.

Data in Fig 1*A–C* describe the dose-related influence of dapsone on neutrophil chemotaxis to FMLP and IL-8, and random motility, respectively. Chemotaxis to FMLP was suppressed by 24% in the presence of 30 $\mu$g/ml dapsone ($p < 0.05$), and 62% in the presence of 300 $\mu$g/ml dapsone. Chemotaxis to IL-8 was decreased by 24% in the presence of 100 $\mu$g/ml dapsone ($p < 0.05$), and 61% in the presence of 300 $\mu$g/ml dapsone. Random motility was decreased by 31% and 35% in the presence of 100 or 300 $\mu$g/ml dapsone ($p < 0.01$).

**Effect of Dapsone on Neutrophil Adherence Function** The inhibitory effect of anti-integrin antibodies on neutrophil chemotaxis under agarose and adherence to protein-coated plastic [17] suggested that dapsone-mediated suppression of chemotaxis might be related to cell adherence, rather than cell functions related to directional motility. Subsequent studies were therefore designed to directly test the influence of dapsone on neutrophil adherence.

Neutrophil adherence function mediated by Mac-1 family integrins was measured in vitro assessing the stimulated binding of cells to albumin coated wells of a microtiter plate, using the tripeptide chemoattractant (FMLP) or phorbol myristate acetate (PMA) as stimulus. Each well contained $2 \times 10^5$ neutrophils in combination with the stimulus, with or without dapsone. After a 60-min incubation period at 37°C, non-adherent cells were removed by washing, and the adherent cells were stained with crystal violet dye and washed. The dye was released from the adherent cells with detergent, and the solubilized dye measured spectrophotometrically at 590 nm using an automated plate reader. The lower limit of this assay was 25,000 cells per well, yielding an optical density (OD) value of ~0.2; 200,000 cells per well, representing the total cells routinely used in the assay, produced an OD value of ~1.2. The change in OD per 25,000 cells averaged ~0.2. The role of Mac-1 family receptors in this adherence assay was established by the ability of an anti-receptor (CD18) antibody to totally block-stimulated neutrophil adherence to the albumin-coated wells (data not shown).

Data from five experiments to assess the effect of dapsone on neutrophil adherence are summarized in Fig 2. In the absence of both stimulus and dapsone, stained adherent cells produced an OD of $0.22 \pm 0.05$ (mean ± SEM). Stimulation with PMA or FMLP in the absence of dapsone increased cell adherence to produce OD values of $0.87 \pm 0.1$ and $0.75 \pm 1.1$, respectively. Dapsone at doses of 10 to 200 $\mu$g/ml did not significantly alter neutrophil adherence in the absence of stimulus but, when present with either stimulus at doses ≥ 50 $\mu$g/ml, produced a dose-related inhibitory effect on adherence. Dapsone at 50 $\mu$g/ml reduced stimulated adherence to 42 and 49% of the respective control values ($p < 0.05$); at 200 $\mu$g/ml, stimulated adherence was reduced to 23 and 8% of these values ($p < 0.01$). The 50% inhibitory doses were approximately 150 $\mu$g/ml for both stimuli. This inhibitory influence was not attributable to the dimethylsulfoxide used to dissolve dapsone, because the solvent at a concentration equal to that present with the highest dose of dapsone (0.2%) did not influence the adherence assay. The negative influence of dapsone on cell adherence was also not attributable to suppression of dye uptake by adherent cells.

**Mechanism of Inhibition of Neutrophil Adherence by Dapsone** The cytotoxicity of dapsone in neutrophils was measured by two methods. A direct assay of cell death involved measurement of the number of cells staining with the fluorescent dye, propidium iodide [25]. Following incubation in tissue culture medium or medium containing 1 $\mu$m FMLP and 200 $\mu$g/ml dapsone for 30 or 60 min at 37°C, cells unable to exclude dye were counted by flow cytometry. Two percent of cells stained with propidium iodide following incubation in control medium for either time period. The number of stained cells increased to 3.3 and 4.6% after exposure to FMLP + dapsone for 30 and 60 min, respectively. This decrease in the viability of neutrophils exposed to stimulus and dapsone was considered too small to account for the inhibitory effect of dapsone on neutrophil-adherence function.

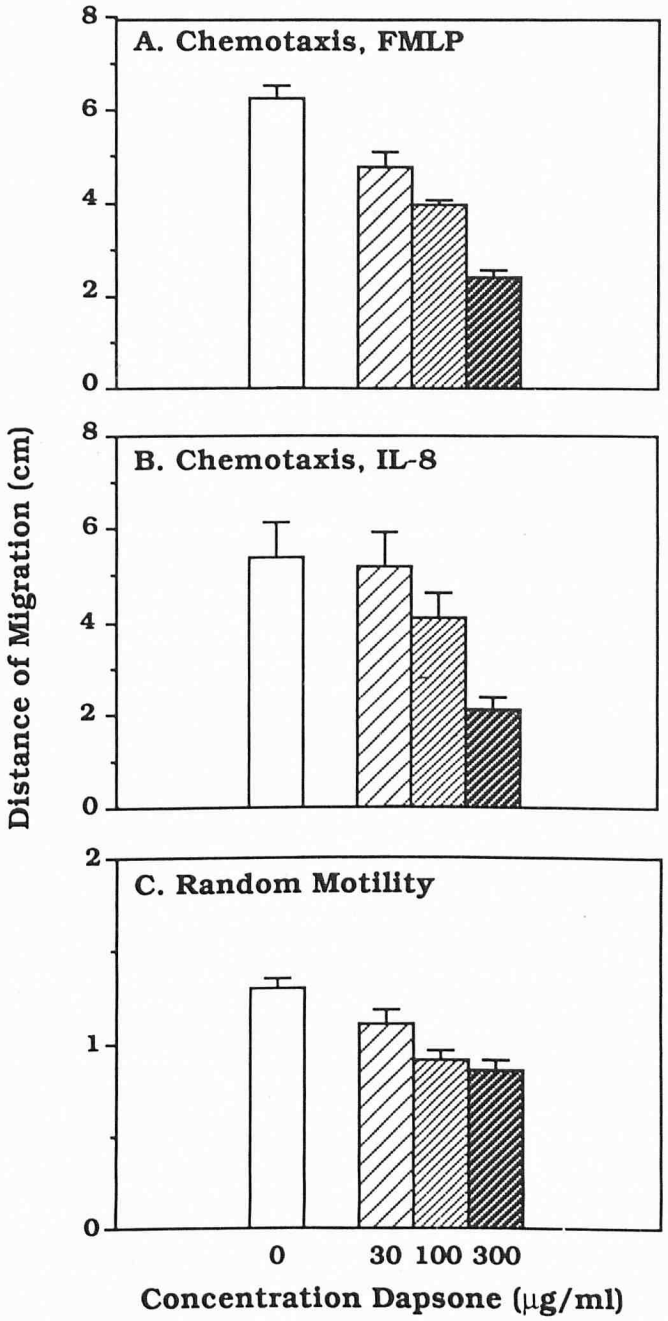


**Figure 1.** Effect of dapsone on human neutrophil migratory functions. Chemotactic and random migratory functions were measured in the under agarose assay. Distances of migration were measured from a projected image magnified approximately 40×. Attractants included N-formylmethionyl-leucyl-phenylalanine (FMLP) and interleukin-8 (IL-8). Dapsone was present in the cell-suspension medium and the agarose gel. Mean ± SEM values derive from three independent experiments.

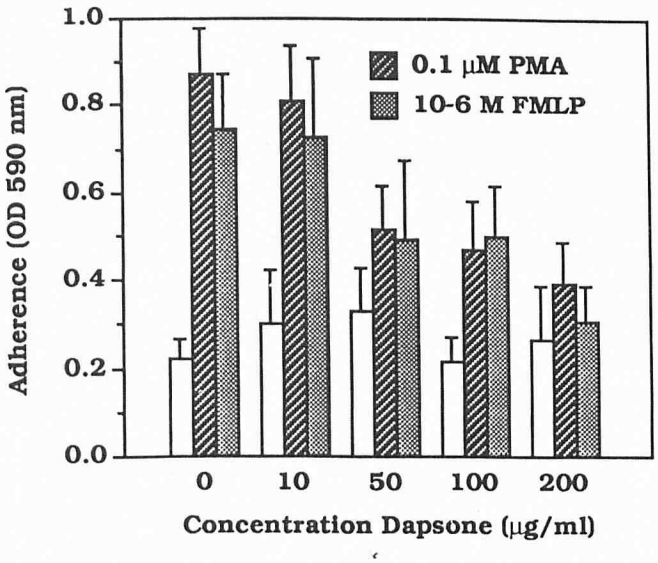


**Figure 2.** Effect of dapsone on human neutrophil adherence function. Stimulants of adherence to albumin-coated wells of microtiter plates were phorbol myristate acetate (PMA) and N-formylmethionyl-leucyl-phenylalanine (FMLP). Adherence was measured spectrophotometrically in terms of uptake of crystal violet dye. Mean ± SEM values derive from five independent experiments.

An indirect assay of dapsone cytotoxicity involved testing the effect of a pulse exposure of neutrophils to dapsone on the subsequent ability of neutrophils to undergo stimulated adherence. In three independent experiments, cells pre-exposed to 200 μg/ml dapsone for 60 min and washed once before transfer to the adherence assay displayed no loss of ability to adhere to the albumin-coated wells in response to PMA or FMLP. These results establish that pre-exposure to dapsone does not functionally disable the neutrophils. A cytotoxic effect of dapsone or DMSO cannot therefore explain the inhibitory influence of dapsone on neutrophil adherence. These results also indicate that the inhibitory influence of dapsone on neutrophil adherence in vitro requires the presence of drug during stimulation and initiation of adherence.

An alternative explanation for the inhibitory effect of dapsone on neutrophil adherence is that dapsone inhibits stimulation by interfering with the binding of agonists. This possibility is supported by evidence that dapsone has a partial inhibitory effect on FMLP binding [3], and would explain the requirement for the presence of drug during stimulation. We have not directly assayed the influence of dapsone on PMA binding, but have tested the effect of dapsone on PMA-stimulated mobilization of Mac-1 receptors. These receptors are transferred from a cytoplasmic storage pool associated with lysosomal granules when cells are stimulated to degranulate [26].

Mac-1 receptor expression was assayed by flow cytometry of neutrophils stained with fluorescein-conjugated Mo1 antibody (specific for the alpha-subunit of Mac-1 receptors) and recorded as mean channel fluorescence (MCF) on a linear scale. The MCF value for cells incubated for 60 min in the absence of either PMA or dapsone was 148 ± 27 (SD), and that for cells incubated with 200 μg/ml dapsone alone was 153 ± 23. These results establish that dapsone does not interfere with either binding of this antibody to Mac-1 receptors or constitutive expression of these receptors. The MCF value for cells incubated with 100 ng/ml PMA alone increased to 250 ± 36, and that for cells incubated with both PMA and dapsone increased to 280 ± 27. The latter results imply that suppression of integrin-mediated neutrophil adherence by dapsone can involve a mechanism unrelated to inhibition of binding of the stimulus.

**Effect of Related Agents on Neutrophil Adherence** Data in Fig 3*A*–*C* describe the effects of sulfapyridine and two structurally related compounds on stimulated neutrophil-adherence function. All agents were tested over the dose range of 10 to 200 μg/ml. Sulfapyridine reproduced the inhibitory influence of dapsone on PMA-stimulated neutrophil adherence, but neither furosemide nor hydrochlorothiazide suppressed this function. These results establish chemical specificity for sulfone-mediated suppression of neutrophil adherence and begin to provide clues to the structural elements involved.

**Effect of Dapsone Therapy on Neutrophil Adherence Function Ex Vivo** The doses of dapsone required to influence neutrophil functions in vitro exceed the peak circulating levels of this drug

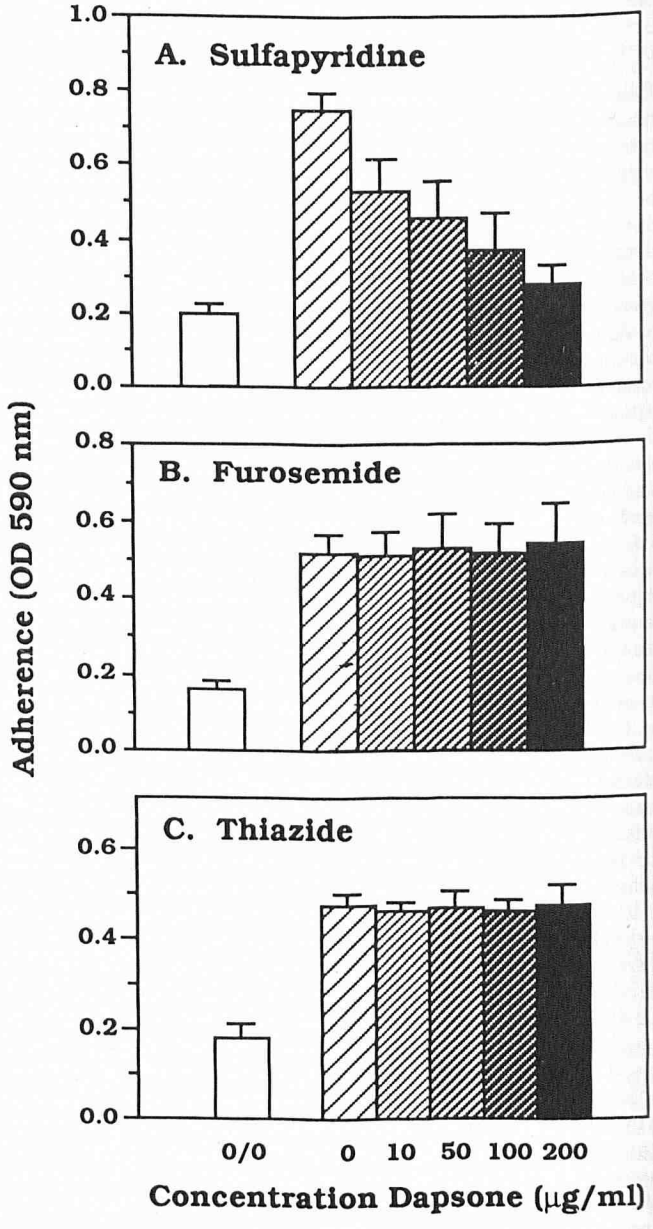


**Figure 3.** Effect of three sulfone analogs on neutrophil adherence. Stimulation of adherence to albumin-coated wells of microtiter plates was achieved with 0.1 μM PMA. Adherence was measured spectrophotometrically in terms of uptake of crystal violet dye. Mean ± SEM values derive from three independent experiments using sulfapyridine and hydrochlorothiazide, and four independent experiments using furosemide.

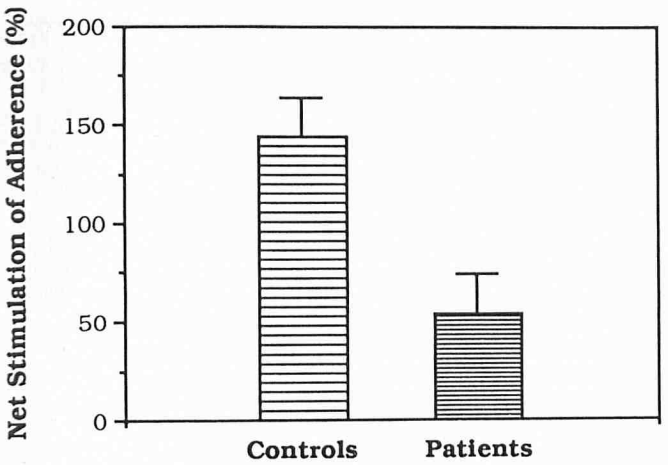


**Figure 4.** Effect of dapsone therapy on neutrophil adherence ex vivo. Neutrophils were isolated from patients with dermatitis herpetiformis receiving 50–200 mg dapsone per day. Stimulation of adherence to albumin-coated wells of microtiter plates was tested using 0.1 $\mu$M PMA. Adherence was measured spectrophotometrically in terms of uptake of crystal violet dye and expressed as net stimulation of adherence in percent. Mean ± SEM values derive from three control donors and three patients.

of 2 to 7 $\mu$g/ml [27,28] by a factor of 10 to 100. One might argue that the in vitro effect of dapsone we have observed is therefore artifactual and unrelated to the clinical anti-inflammatory influence of the drug. To consider this relationship, we additionally evaluated the in vitro adherence function of neutrophils isolated from three patients with dermatitis herpetiformis taking either 50 mg or 200 mg of dapsone daily.

Data summarized in Fig 4 describe stimulated adherence functions of neutrophils isolated from three patients with dermatitis herpetiformis. Background levels of adherence of patient neutrophils were normal (data not shown), but addition of PMA to stimulate adherence did not produce a normal increase in adherence function. Net stimulated adherence of patient cells averaged 44% of background versus 144% for control cells. We therefore conclude that dapsone treatment of neutrophils in vitro reproduces the effect of dapsone medication on an integrin-mediated adherence function of neutrophils and that the in vitro effect of dapsone must be relevant despite the higher dose requirement.

## DISCUSSION

Dermatitis herpetiformis rapidly responds to treatment with dapsone or sulfapyridine likely involving the anti-inflammatory property of these drugs. The absence of neutrophils in the skin of sulfone-treated patients [3] suggests that these drugs might act by suppressing neutrophil recruitment to lesional sites. Failure of sulfones to influence the deposition of IgA or complement, including C5, in skin [11,12] indicates that therapy does not abrogate the production of complement-derived chemoattractants. Sulfone-mediated suppression of neutrophil chemotaxis to FMLP and $LTB_4$ have been demonstrated experimentally in vitro [2–4], but the chemoattractants present in DH are likely not limited exclusively to these agents.

We suggest that a more basic mechanism contributes to suppression of neutrophil emigration following sulfone therapy, namely, drug-mediated inhibition of neutrophil adherence to vascular endothelium. Adherence reactions are a requisite step in leukocyte recruitment to extravascular sites in response to multiple chemoattractants. Adherence to endothelium involves several neutrophil receptor species acting in sequence to initiate cell accumulation and support the crawling style of leukocyte motility. In one scheme, the selectins provide for targeting of leukocytes to sites of inflammation, and integrins mediate the adherence necessary for neutrophils to move on and through the vascular endothelium [29]. The essential role of the CD11/CD18 family of integrins, which includes Mac-1, LFA-1, and p150,95 receptors, is definitively supported by the failure of patients genetically unable to synthesize these receptors to recruit neutrophils to sites of infection [17,30]. Suppression of integrin-mediated adherence by sulfones would therefore similarly prevent circulating neutrophils from emigrating to extravascular sites.

Neutrophil adherence mediated by Mac-1 family receptors can be measured in vitro by assaying binding of stimulated cells to protein-coated glass or albumin-coated latex beads [17]. The role of these integrins in this assay can be demonstrated by the ability of various anti-receptor antibodies to block stimulated adherence: stimulated adherence is most effectively inhibited by an anti-beta subunit (CD18) antibody followed, in order, by antibodies to the alpha subunits of Mac-1, p150,95, and LFA-1 [17]. A similar rank order was observed for suppression of neutrophil chemotaxis under agarose by these antibodies, but these antibodies did not alter neutrophil chemotactic function in a membrane filter assay. The relevance of these assays to integrin-mediated neutrophil adherence functions in vivo is supported by observations that adherence abnormalities induced by antibodies in vitro are also observed with neutrophils from patients with leukocyte adherence deficiency [16], and that administration of anti-beta subunit antibodies to mice or rabbits compromises neutrophil recruitment [18–20].

The possibility that dapsone might interfere with neutrophil adherence was first suggested by our findings that dapsone suppressed neutrophil random motility, as well as chemotaxis to two chemoattractants, in the integrin-dependent under-agarose assay (Fig 1). Subsequent tests confirmed that dapsone and sulfapyridine do inhibit integrin-mediated binding of neutrophils to albumin-coated plates (Figs 2 and 3). These observations, together with evidence that Mac-1 family receptors are not required for neutrophil chemotaxis through membrane filters [17], would indicate that dapsone may have different mechanisms of action in different assays of chemotaxis. In the membrane filter system, dapsone may inhibit chemotaxis only to attractants with which it interferes with binding [3,31]; in the under-agarose system, dapsone may inhibit chemotaxis to multiple attractants by suppressing attractant-stimulated cell adherence to the plate surface. Different dose requirements for these alternative effects of dapsone may then explain earlier failures of some investigators to find an inhibitory influence of dapsone on neutrophil chemotaxis under agarose [6,24].

The 50–200 $\mu$g/ml doses of dapsone required to suppress neutrophil migratory adherence function were an order of magnitude higher than plasma levels of the drug [27,28]. The high concentrations of dapsone required to affect these cell functions in vitro could be taken to suggest that inhibition of adherence in vitro is not relevant to mechanisms of action of dapsone in vivo. Our observation that neutrophils from three individuals receiving ordinary doses of dapsone (50 mg/d and 200 mg/d) did not adhere normally to the albumin-coated plates, however, provides strong support for the clinical relevance of our findings. We speculate that the dose discrepancy between in vitro and in vivo effects of dapsone on neutrophil adherence reflects the role of drug metabolites [31], contributions of plasma components, and/or differences in time of exposure to the drug.

We have yet to discover how sulfones suppress the adherence function of Mac-1 family integrins. We do have several clues, however, to indicate how dapsone does not work. Using flow cytometry in conjunction with Mo-1 anti–Mac-1 antibody, we have determined that 200 $\mu$g/ml dapsone does not influence either binding of this anti-alpha subunit antibody or stimulated mobilization of Mac-1 receptors to the cell surface. Thus, sulfones appear more likely to interfere with receptor-ligand interaction or activation of the adherence function of Mac-1 receptors [17]. An influence on activation is suggested by the apparent role of oxidation in Mac 1–dependent cell adhesion [31] and the ability of neutrophils to oxidize an $-NH_2$ group present on dapsone and sulfapyridine [32]. Whatever the mechanism, we must also characterize the effects of sulfones on neutrophil adherence to counter receptors on the surface of vascular

endothelial cells [33,34]. Although these questions remain, the evidence presented here certainly provides preliminary support for dapsone and other sulfones serving as "anti-adherence" therapeutic agents in dermatoses involving inappropriate accumulation of leukocytic phagocytes.

## NOTE ADDED IN PROOF

Since this manuscript was submitted, we have found that dapsone suppresses neutrophil adherence stimulated by two additional agonists, C5a, the activated complement-derived chemoattractant prepared as zymosan-activated serum, and tumor necrosis factor alpha. C5a is likely a chemoattractant in dermatitis herpetiformis. These findings also provide additional evidence that suppression of stimulated neutrophil adherence by dapsone is agonist independent. A recent abstract by V. Thuong-Nguyen and J.J. Zone [J Invest Dermatol 96:556A, 1991] demonstrates suppression of neutrophil adherence to IgA, identifying still another neutrophil-adherence function affected by this agent.

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